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MONITORING FOR COMMERCIAL BROILER BREEDER FLOCKS AND THEIR PROGENY FOR MYCOPLASMA GALLISEPTICUM INFECTION AND PERFORMANCE AFTER VACCINATION WITH TS-11

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ABSTRACT

Monitoring and comparison of MG natural infection occurrence, vertical transmission, egg production and hatchability between same breed two sequential flocks in three broiler breeders farms were performed. The first flock in each farm used different Mg control programs as killed vaccines, antimycoplasmal drugs, live vaccine (F strain) or mixed program (as killed vaccine with antimycoplasmal drug) and the next flock in each farm used TS-11 program (Vaxsafe MG™, Bioproperities®) administrated by eye drop at age of 3-4 weeks after performing ELISA test to ensure that flocks are free from MG antibodies before vaccination. MG natural infection occurrence and vertical transmission were assessed by PCR for detection of field strain in breeders lower trachea, ovaries and air sacs and their progeny clonal cavity and upper trachea. Egg production and hatchability were assessed by comparison of weekly production and hatchability with ideal breed production and hatchability catalog curves and curves of previous flocks used other programs for controlling MG.TS-11 gave better results counter to other MG control programs in all parameters where no natural infection or vertical transmission has been detected where all collected samples gave negative results for PCR except one sample in farm (A) and when repeating sampling and PCR test at 48 weeks old it gave negative result which explaining exposure to challenge but no infection occurs and performance was the best with TS-11 where production and hatchability was better and the differences were significant.

Key words: Mycoplasma gallisepticum, TS 11, Vaccination

INTRODUCTION

In Egypt Mycoplasma gallisepticum (MG) is the most economically significant mycoplasmas species in poultry industry. Mycoplasmas are among the most important egg-borne diseases in poultry industry. Spreading of infection in breeder and layer leads to high losses during the production cycle. It can be transmitted by both lateral and vertical routes (Ley, 2003).

The best approach for controlling mycoplasma is depopulation of the infected sites followed by cleaning and disinfection and 4-6weeks free period. This approach is very difficult and may be impossible in our country due to economic issues. So, practically, shifting from eradication to control is necessary. Control program depend on one of this after a diligent biosecurity: 1-Medication with antimycoplasmal drugs.2-Vaccination (Live, Killed vaccines and vector vaccines).

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Medication using antimycoplasmal drugs including Macrolides, Tetracyclines and Flouroquinolonesis is useful (Ley, 2003). Killed vaccines (Oil emulsion) reduce infection but usually tracheal colonization by Mycoplasma gallisepticum after challenge with virulent strains occurs (Yagihashi *et al.*, 1992). Live vaccine strains used for MG controlling are F. Strain, 6/85 and TS-11. F. Strain and 6/85 (originated from USA) and Ts-11(originated from Australia), are used for controlling of MG infection in several countries (Ley, 2008). Temperature sensitive mutant strains are able to colonize in the upper respiratory tract. They are not able to survive at the temperature of the lower tract and air sacs (Levisohn *et al.*, 1987). Recombinant fowl pox virus – MG (Rfpv –MG) is used for protection against MG and give better results when used with F. Strain (Pakpinyo *et al.*, 2015).

PCR and recently real time PCR allows rapid detection of MG from cultures or directly from clinical samples (Callison *et al.*, 2006).

TS-11 can't survive in temperature more than 33˚c so samples taken from lower trachea, lung and air sacs help for direct detection of field MG without vaccinal strain detection.

Aim of study

In Egypt many broiler breeder farms were exposed to MG infection which causes significant losses in egg production and hatchability in addition to transovarian transmission which badly affect the offspring performance despite using different available control programs. Newly introduced TS-11 MG live vaccine was a chance to evaluate it under field conditions in farms already have been exposed for infection for its efficacy for prevention of infection, prevention of vertical transmission and flocks performance.

MATERIALS AND METHODS

Samples sources:

Six flocks, of same breed and two sequential flocks in three broiler breeders farms

Three Farms data:

These have been coded (A,B and C) and each one of the two flocks in each farm was coded (TS AND O) as below:

ATS = flock in farm A Vaccinated with TS-11
AO = flock in farm A use other program for controlling MG
BTS = flock in farm B Vaccinated with TS-11
BO = flock in farm B use other program for controlling MG
CTS = flock in farm C Vaccinated with TS-11
CO = flock in farm C use other program for controlling MG

Table 2.1: Showing data of the six flocks of the three farms.

		ATS	AO	BTS	BO	CTS	CO	NOTES
Date of Housing		03/01/2015	12/04/2013	27/03/2015	9/06/2013	22/05/2015	22/08/2013	
No of birds	♀	20000	20000	30000	30000	20000	20000	
	♂	3000	2600	4300	4000	2800	2500	
Breed		Cobb 500 FF	Cobb 500 FF	Cobb 500 FF	Cobb 500 FF	Cobb 500 FF	Cobb 500 FF	
Area		Alexandria Desert road	Alexandria Desert road	Sharqia	Sharqia	Dakhlia	Dakhlia	

Table 2.2: Showing mycoplasma gallisepticum control program for the six flocks.

	Vaccination			Medication					
	Killed MG vaccine		Live MG Vaccine	1st week	4rd week	8th week	12 week	16week	From 20 week till end of production
ATS			TS-11 at 4 weeks	Doxycycline +colistin					
AO	11 weeks	16 weeks		Antibiotic combination	Antimycoplasma DW	Antimycoplasma DW	Antimycoplasma DW	Antimycoplasma In feed	Antimycoplasma In feed every 3-4 weeks
BTS			TS-11 at 6 weeks	Doxycycline +colistin					
BO			F strain at 8 weeks	Antibiotic combination	Antimycoplasma DW			Antimycoplasma In feed	Antimycoplasma In feed every 3-4 weeks
CTS			TS-11 at 3 weeks	Doxycycline +colistin					
CO				Antibiotic combination	Antimycoplasma DW	Antimycoplasma DW	Antimycoplasma DW	Antimycoplasma In feed	Antimycoplasma In feed every 3-4 weeks

TS-11 vaccine = Vaxsafe MG™, Bioproperities® administrated by eye drop
Killed MG vaccine= NOBILIS MG INAC™ MSD®
F.strain = Cevac MG F ™, CEVA®
Antimycoplasma DW = Tilmicosin or Tylosin
Antimycoplasma in feed = Tylosin
TS-11 vaccine = Vaxsafe MG™, Bioproperities® administrated by eye drop, Killed MG vaccine= NOBILIS MG INAC™ MSD®, F.strain=Cevac MG F™, CEVA®, Antimycoplasma DW=Tilmicosin or Tylosin, Antimycoplasma in feed = Tylosin

Protocol of sampling

A- Before vaccination, samples are Blood.

B-Six weeks post vaccination, samples are organs (Lower trachea, lung, air sacs and ovaries).

C-Prior to laying at 20 weeks of age, samples are organs (Lower trachea, lung, air sacs and ovaries).

D- At peak of production (30 weeks of age), samples are organs (Lower trachea, lung, air sacs and ovaries).

E- From progeny at peak of production (30 weeks of age), samples are Tracheal swabs.

F- At the second phase of production (at 45 weeks of age), samples are organs (Lower trachea, lung and air sacs).

Samples collection:

A-Blood from live birds broiler breeders a week prior vaccination.

B- Lower trachea, lung and air sacs from freshly dead broiler breeder are collected over a week as protocol mentioned.

C-Tracheal swabs from progeny collected from each flock as mentioned.

Samples Data

Table 2.3: Showing data of samples.

Code	Age/W	Breed	No of birds ♀	No of birds ♂	Area	No. of samples	Type of samples	NOTES
ATS 1	3	cobb	19850	2980	Alex. Desert road	20	Blood	
ATS 2	10	cobb	19680	2950	Alex. Desert road	30	LT,L,AS,O	
ATS 3	20	cobb	19450	2920	Alex. Desert road	35	LT,L,AS,O	
ATS 4	30	cobb	18600	2780	Alex. Desert road	38	LT,L,AS,O	
ATS 5	30	cobb			Alex. Desert road	20	TS	From progeny day old
ATS 6	45	cobb	18200	2690	Alex. Desert road	42	LT,L,AS,O	
ATS 7	48	cobb	18100	2675	Alex. Desert road	33	LT,L,AS,O	
BTS 1	5	cobb	29700	4350	Sharqya	30	Blood	
BTS 2	10	cobb	29500	4250	Sharqya	38	LT,L,AS,O	
BTS 3	20	cobb	29200	4180	Sharqya	45	LT,L,AS,O	
BTS 4	30	Cobb	28080	4080	Sharqya	50	LT,L,AS,O	
BTS 5	30	cobb			Sharqya	20	TS	From progeny day old
BTS 6	45	cobb	27200	3900	Sharqya	55	LT,L,AS,O	
CTS 1	2	cobb	19900	2800	Dakhlia	20	Blood	
CTS 2	10	cobb	19750	2750	Dakhlia	28	LT,L,AS,O	
CTS 3	20	cobb	19500	2700	Dakhlia	30	LT,L,AS,O	
CTS 4	30	cobb	18250	2600	Dakhlia	35	LT,L,AS,O	
CTS 5	30	cobb			Dakhlia	20	TS	From progeny day old
CTS 6	45	cobb	17600	2500	Dakhlia	40	LT,L,AS,O	

LT=Lower tracheal part of freshly dead, L=Lung, AS =Air sac, O=Ovaries, TS= Tracheal swab.

Samples transport and storage

A-Blood from live bird's broiler breeders were collected and transported in ice box and stored in refrigerator.

B-Samples are collected daily during aweek from freshly dead and transported in ice box and stored in freezer.

C-Tracheal swabs from progeny were collected and transported in ice box and stored in freezer.

ELISA (Enzyme-linked immunosorbent assay)

Were performed to check Mycoplasma gallisepticum

antibodies before vaccination of flocks with TS-11 live vaccine (VAXSAFE MG, BIOPROPERITIES ®) using Biochek ® Enzyme-linked Immunosorbent Assay (ELISA) for poultry were used as a standardized kit. As manufacturer recommendations.

Real time Polymerase Chain Reaction (real time PCR) Technique

Using pooling strategy (8-12 samples were pooled together in one tube), RNeasy Mini Kit (Qiagen, Valencia, CA, USA) protocol. primers and the probe described as below:

Table 2-4: Showing the primers and probes used in real time PCR.

Primers	Primer 5'-3'	References
MGLPU26-F	5'-CTA GAG GGT TGG ACA GTT ATG - 3'	Callison *et al.,* 2006
MGLP164-R	5'-GCT GCA CTA AAT GAT ACG TCA AA - 3'	
MGLP-P	5'-FAM) -CAGTCATTAACA ACT TAC CAC CAG AAT CTG - (MGB) – 3'	

RESULTS

Table 3-1: Showing Elisa results.

Code	Elisa Results	NOTES
ATS 1	Negative	
BTS 1	Negative	
CTS 1	Negative	

Table 3-2: Showing PCR results.

Code	Real time PCR Results	NOTES
ATS 2	Negative	
ATS 3	Negative	
ATS 4	Negative	
ATS 5	Negative	From progeny
ATS 6	Positive	
ATS 7	Negative	
BTS 2	Negative	
BTS 3	Negative	
BTS 4	Negative	
BTS 5	Negative	From progeny
BTS 6	Negative	
CTS 2	Negative	
CTS 3	Negative	
CTS 4	Negative	
CTS 5	Negative	From progeny
CTS 6	Negative	

Table 3-3: Performance monitoring results.

	ATS	AO	BTS	BO	CTS	CO	NOTES
production peak %	85.5	81.7	87	80	86.4	79.2	
Perseverance on production peak (weeks)	9	5	11	6	11	Not reach 80%	
Cumulative Hatchability %	85.6	78	84.6	79.3	84.4	76	
Total eggs / hen	181.2	173	179.2	168	179	161	
Mortality %	10.3	14	9.2	12.3	10.25	12.4	

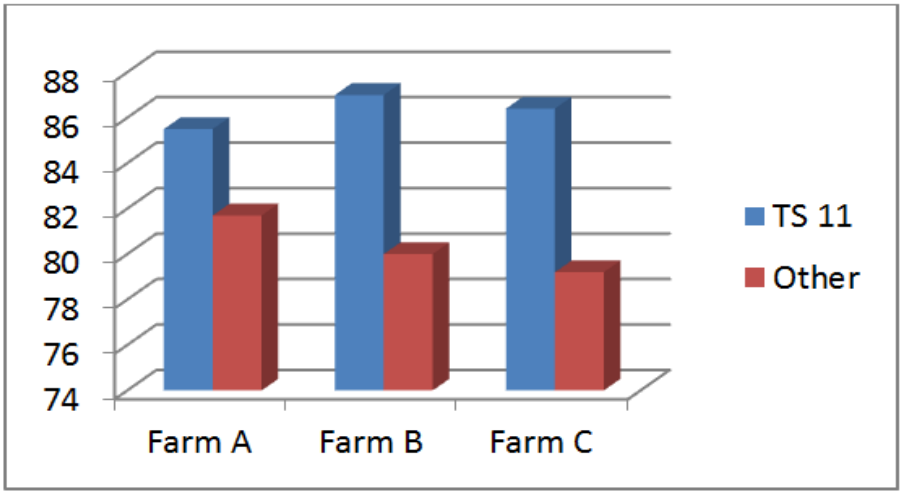

Figure 3.1: Showing production peak% in the six flocks.

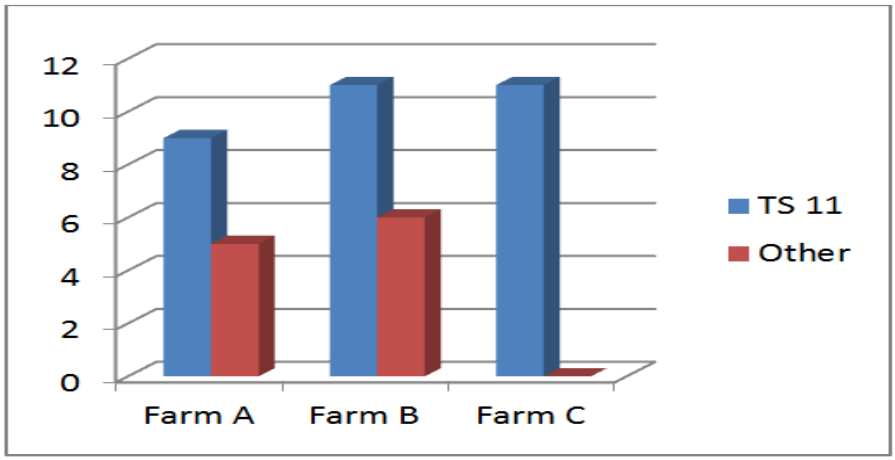

Figure 3.2: Showing perseverance on peak of the six flocks.

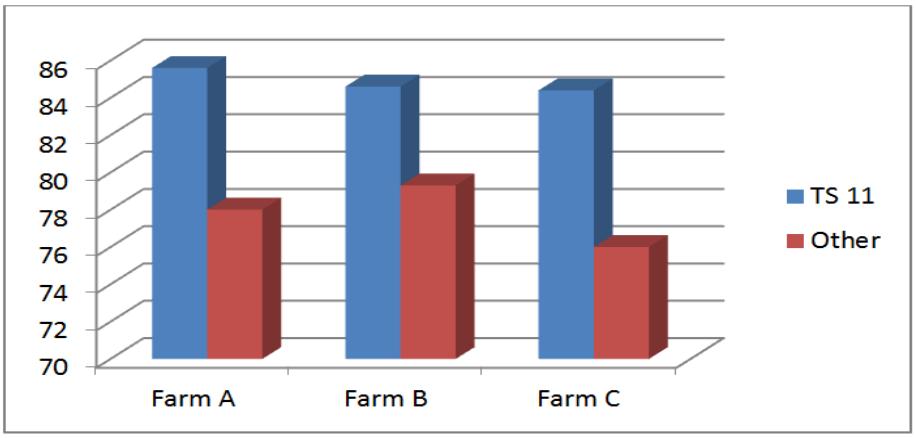

Figure 3.3: Showing cumulative hatchability % in the six flocks.

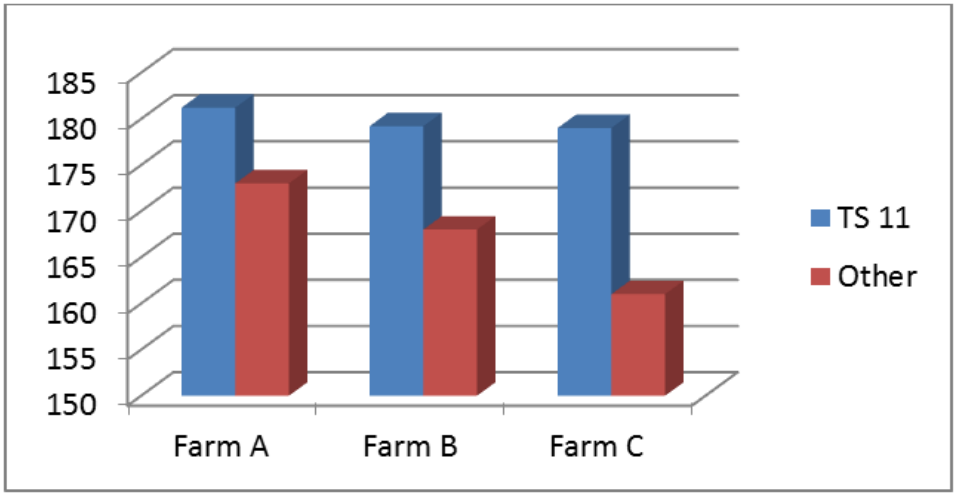

Figure 3.4: Showing total eggs / hen in the six flocks.

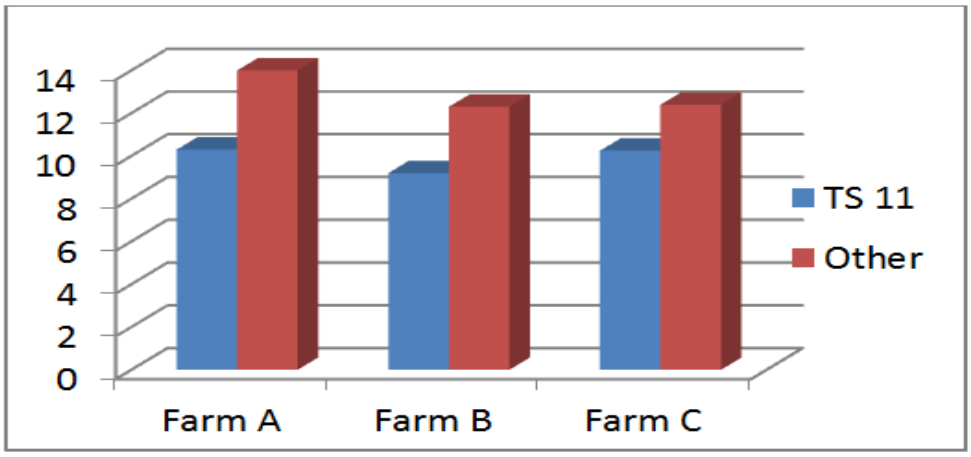

Figure 3.5: Showing Mortality % in the six flocks.

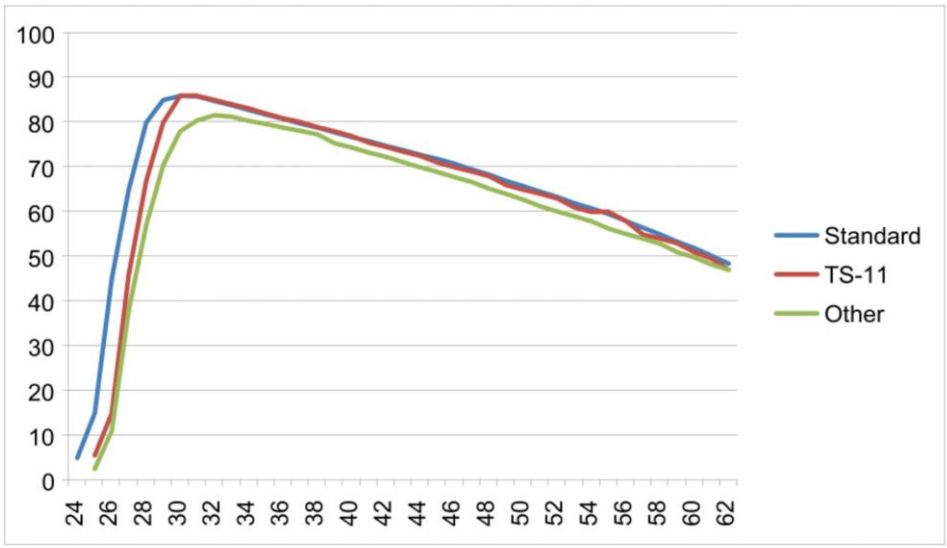

Figure 3-6: Comparison production % per week between two flocks in farm A with standard and each other:

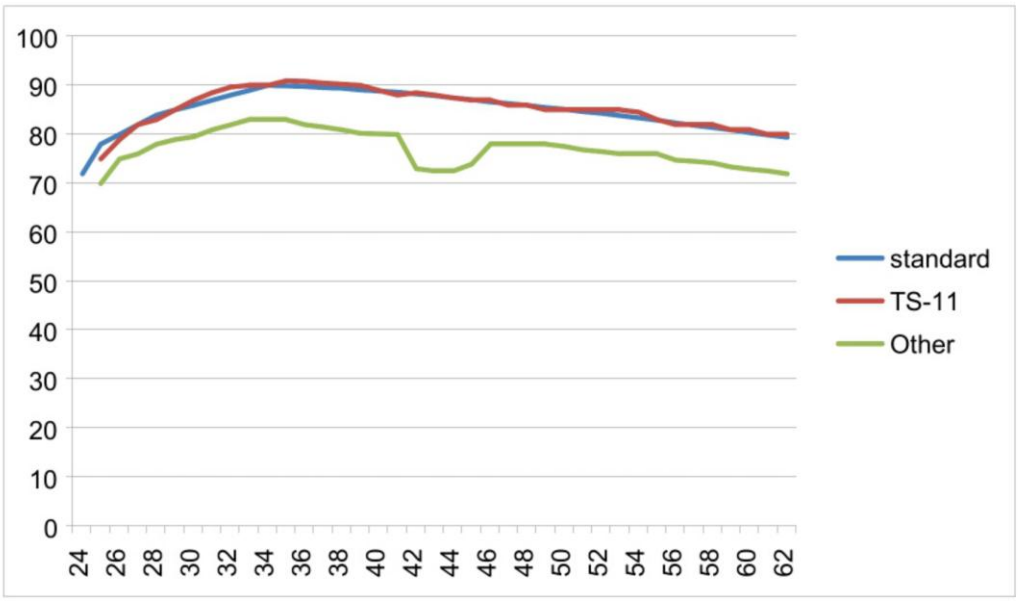

Figure 3-7: Comparison hatchability % per week between two flocks in farm A with standard and each other:

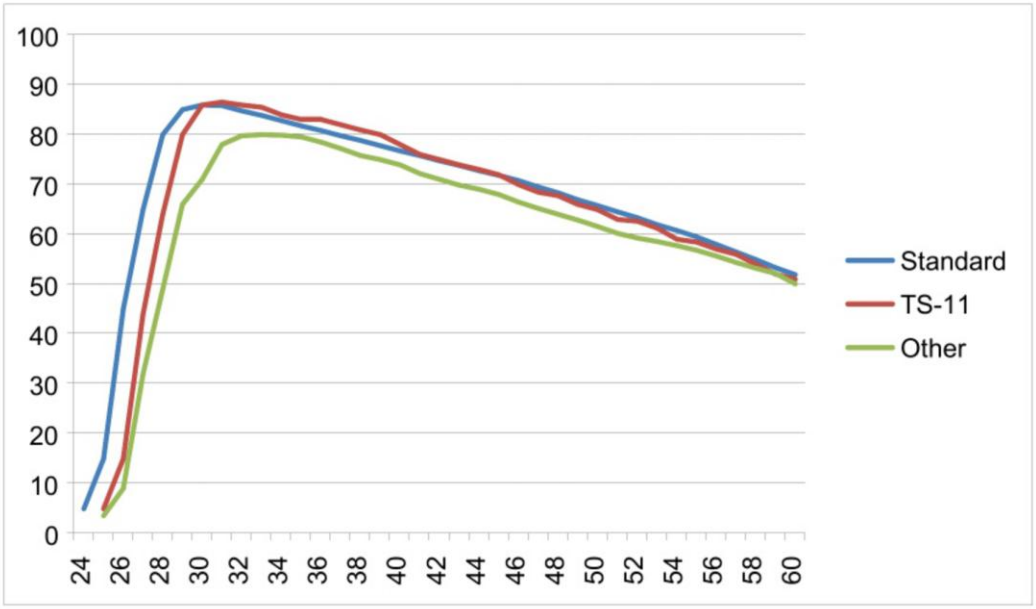

Figure 3-8: Comparison production % per week between tow flocks in farm B with standard and each other:

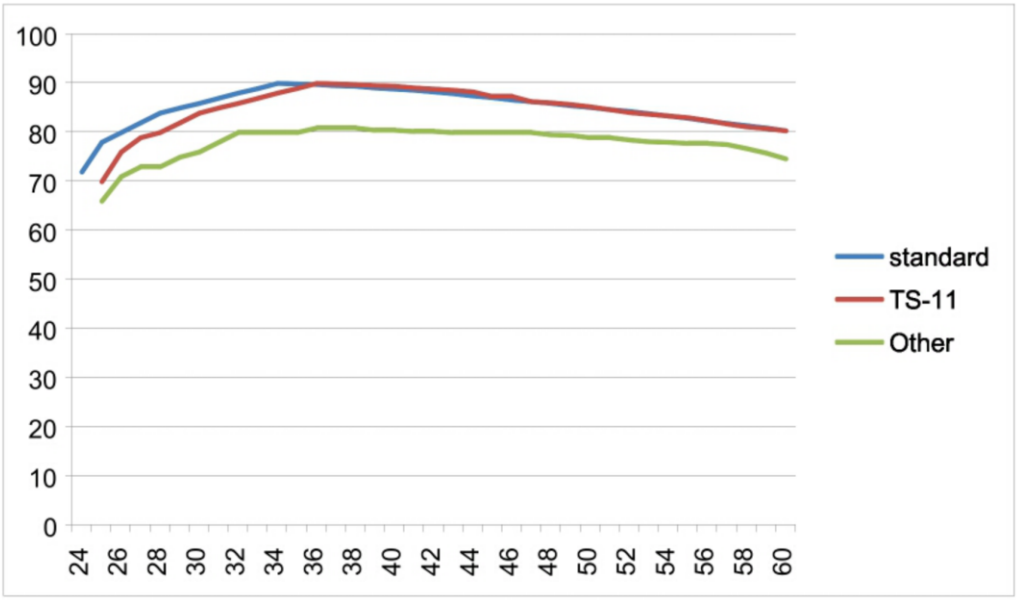

Figure 3-9: Comparison hatchability % per week between two flocks in farm B with standard and each other:

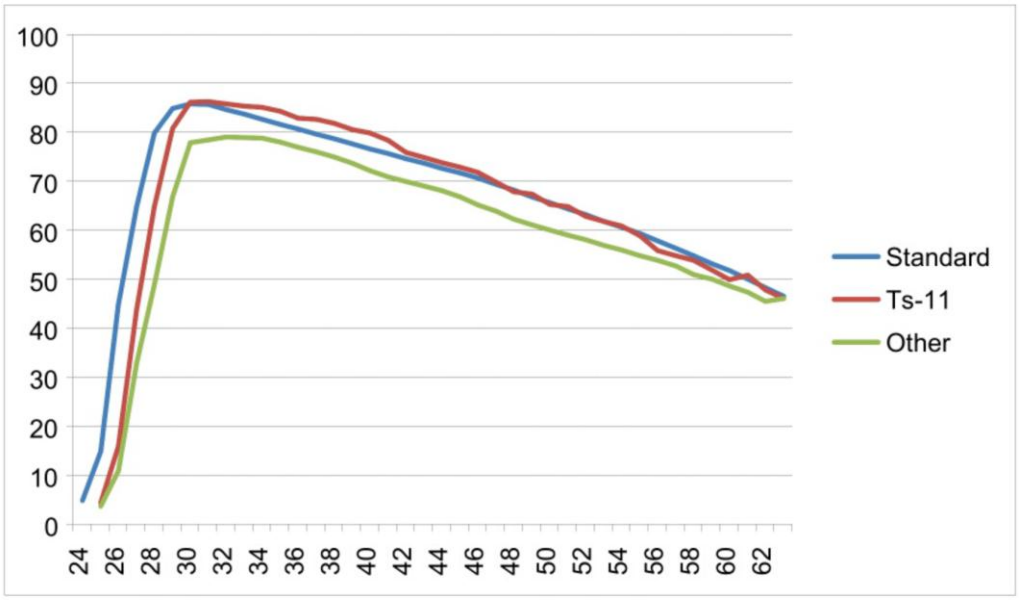

Figure 3-10: Comparison production % per week between two flocks in farm C with standard and each other:

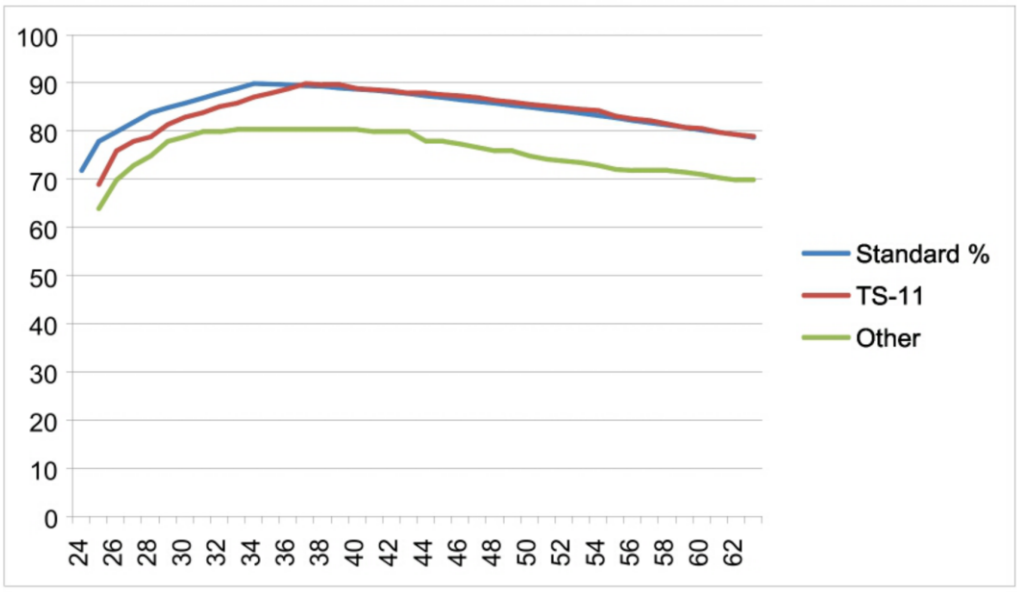

Figure 3-11: Comparison hatchability % per week between two flocks in farm C with standard and each other:

DISCUSSION

Ts-11 vaccine has been determined as low virulent or avirulent strain and has extensively been used as atoll for the control of Mycoplasma gallisepticum since its introduction in Australia 20 years ago (Bioproperities®, 2005). And has been showed to provide protection against respiratory disease, egg production losses and vertical transmission associated with virulent Mycoplasma gallisepticum infection (whithear *et al.*, 1990a; Abd- elmotelib and Kleven, 1993; Whithear 1996; Barbour *et al.*, 2000).

The majority of laboratory studies showed that vaccinated chickens with ts-11 strain have been able to resist challenge with virulent M. gallisepticum administered by direct air sac inoculation, aerosol (Whithear *et al.*, 1990a) or intra-tracheal inoculation (K.G. Whithear, 1996). Protection against respiratory signs persists up to at least 40 weeks after vaccination (Whithear and Harrigan, 1993), and was significantly greater than the protective immunity elicited by bacterin (Whithear and Harrigan, 1994). In experimental challenge studies, the ts-11 strain almost invariably provides solid protection against the development of tracheal and nasal sinus lesions but protection against the development of air sac lesions is less consistent (Whithear and Harrigan, 1993).

A comparative study by Abd-El-Motelib and Kleven (1993) showed that F strain provided higher protection against the development of air sac lesions following aerosol challenge than that of the milder vaccine strains 6/85 and ts-11. However, using inoculation into the infraorbital sinuses as a method of challenge, ts-11 gave a higher protection than that of both F and 6/85 strains.

Our study results showed the ability of Ts-11 vaccine to protect chickens from natural infection of MG till 45 weeks old (results of PCR as shown in table 3-2) and show the efficacy of Ts-11 in comparison with other programs used previously in these farms for MG control. Only one sample in farm (A) was positive and when repeating sampling and PCR test at 48 weeks old gave negative result which explaining exposuring to challenge but no infection occurs. This is in agreement with and ensure results of whithear *et al.* (1990a); Abd- elmotelib, Kleven, (1993); Whithear (1996). This reported a variable efficacy of Ts-11 in challenge experiments.

Vaccination with F strain significantly reduced the rate of and delayed, but did not eliminate, egg transmission of M. gallisepticum in hens challenged by aerosol (Glisson and Keleven, 1984). Similarly, ts-11 strain reduced but did not prevent egg transmission in hens challenged by direct inoculation of virulent M. gallisepticum into the abdominal air sacs (K.G. Whithear, 1996). Elgazzar *et al.* (2011) report that Ts-11 vaccine was transmitted to egg through ovaries. In this study results of examination of tracheal swabs of day old chicks produced from vaccinated breeders of all three farms were negative for MG filed or vaccine strain (results of PCR are shown in table 3-2) and this revealed the ability of Ts-11 to prevent vertical transmission of MG to offspring our result is in agreement and ensures results of (Whithear 1996 and

Barbour *et al.,* 2000). And disagree these (Elgazzar *et al.,* 2011).

A mathematical model was designed to evaluate the effect of Mycoplasma gallisepticum live vaccines on preventing drops in egg production in layers (Evans and Hafez, 1992) Hens vaccinated with strain 6/85 produced significantly fewer ($P < 0.05$) eggs than those vaccinated with F strain in the periods immediately after challenge (i.e. deterioration and early recovery phases), but significantly more ($P < 0.05$) in the late recovery phase. Pullets vaccinated with ts-11 produced 41.3 eggs over a seven-week period after air sac inoculation with virulent M. gallisepticum, compared with 25.8 eggs ($P < 0.01$) produced by unvaccinated hens. There was no significant difference in the number of eggs laid by vaccinated hens after challenge and those laid by a group of unchallenged controls (Whithear *et al.,* 1990). In six field trials conducted prior to the commercial introduction of ts-11 into Australia, the mean production of vaccinated flocks was 219.6 eggs per hen housed to 65 weeks of age, compared with 211.9 eggs for unvaccinated groups ($P < 0.01$) (Whithear and Harrigan, 1994).

In this study Egg production and hatchability results specially till 45 weeks old was better than ideal curves and in comparison with the previous flocks which use other programs and were complained from infection the results are significantly differ.

Total eggs / hen in farm A using other program was 173 and when using TS_11, it reached 181.2 also in farm B it was 168 and reach 179.2 and in farm C it was 161 and reached 179 that was significantly more ($P < 0.05$). These results ensure results of (whithear *et al.,* 1990a; Abd- elmotelib and Kleven, 1993; Whithear, 1996).

Our study shows the ability of Ts-11 to protect chickens from natural infection of MG, prevent vertical transmission to the progeny and also keeping egg production and hatchability not affected.

Ts-11 vaccine is recommended for using in highly endemic areas and considered the safest and most efficient live vaccine for controlling of MG infection where total eradication is considered impossible.

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متابعة قطعان أمهات التسمين والكتاكيت الناتجة منها لعدوى الميكوبلازما جاليسيبتكم وأداء القطعان بعد تحصينها بلقاح ميكوبلازما تى إس –١١

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فى هذه الدراسة أجريت متابعة فى قطيعيين متتابعين من نفس السلالة فى ثلاثة مزارع لأمهات التسمين للمقارنة بين برامج مختلفة لمقاومة الميكوبلازما جاليسيبتيكم لتقييم حدوث العدوى الطبيعية بالميكوبلازما جاليسيبتيكم وإنتقالها للأجنة وإنتاج البيض والفقس. واستخدم القطيع الأول في كل مزرعة برامج مختلفة لمقاومة الميكوبلازما جاليسيبتيكم كاللقاح الحى عترة إف ، اللقاحات الميتة، مضادات الميكوبلازما أو برنامج مختلط والقطيع التالى في كل مزرعة استخدم برنامج لقاح تي إس -١١ (فاكس سيف ®) بالتقطير فى العين في عمر ٣-٤ أسابيع بعد إجراء اختبار فحص الإنزيم المرتبط المناعى للتأكد من أن القطعان خالية من الأجسام المضادة للميكوبلازما جاليسيبتيكم قبل التحصين. تم تقييم كل من حدوث العدوى الطبيعية وإنتقالها للأجنة عن طريق أختبار البلمرة المتسلسل العكسى للكشف عن العترة الحقلية في أمهات التسمين فى الجزء السفلى من القصبة الهوائية ،المبيض والأكياس الهوائية وفى الكتاكيت الناتجة فى القصبة الهوائية والتجويف الحلقى. تم تقييم إنتاج البيض ومعدل الفقس من خلال مقارنة منحنيات الإنتاج الأسبوعي والفقس للقطعان مع منحنيات السلالة المثالية ومنحنيات القطعان السابقة التى استخدمت برامج أخرى للسيطرة على الميكوبلازما جاليسيبتيكم. أعطى تي إس-١١ نتائج أفضل مقابل البرامج الأخرى حيث لم يتم الكشف عن أي عدوى طبيعية أو انتقال للكتاكيت عن طريق البيض وكل العينات المسحوبة من القطعان المحصنة كانت سلبية فى إختبار البلمرة المتسلسل العكسى ماعدا عينة واحدة فى مزرعة (أ) فى عمر ٤٥ أسبوع كانت إيجابية وعند إعادة سحب العينات والإختبار كانت النتيجة سلبية دلالة على حدوث تعرض للميكروب مع عدم حدوث عدوى وكان الأداء الأفضل مع تي إس-١١حيث أن معدلات الإنتاج والفقس كانت أفضل وكانت الفروق معنوية.